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Differential effects of estrogens and progestins on the anticoagulant tissue factor pathway inhibitor in the rat

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Abstract

Oral contraceptives (OC) and postmenopausal hormone therapy (HT) modulate plasma levels of proteins that regulate blood coagulation. It remains unclear whether the progestin component contributes to these changes. The present study was designed to determine whether progestins modulate two essential plasma anticoagulants, antithrombin (AT) and tissue factor pathway inhibitor (TFPI), in an animal model. Ovariectomized rats were treated orally with three progestins, norethindrone acetate (NETA), trimegestone (TMG), or drospirenone (DSP), either alone or combined with 17α-ethyinylestradiol (EE). Plasma AT levels were unchanged. However, TFPI activity was reduced by EE alone (10–100 μg/kg/day) in a dose-dependent manner; NETA (3 or 10 mg/kg/day) reduced TFPI by ∼40 or ∼80%, respectively, while TMG and DSP had no effect. NETA and EE effects were blocked by co-administration of ICI-182,780, an estrogen receptor antagonist, suggesting that both responses were likely estrogen receptor-mediated. Reduced TFPI after NETA or EE treatment was not accompanied by changes in TFPI mRNA levels in tissues that express TFPI, but there was a positive correlation between plasma TFPI and total cholesterol. Sex hormone effects on TFPI in this model and as reported in women may help to shift the coagulation balance to a more prothrombotic state. Progestins such as TMG and DSP that lack estrogenic activity could potentially have an improved clinical profile.

Keywords: Progestin; Trimegestone; Drospirenone; Norethindrone acetate; Estrogen; Hormone replacement therapy; Tissue factor pathway inhibitor; Thrombosis; Coagulation

1. Introduction

Progesterone is a steroid hormone that has a crucial role in the regulation of female reproduction. Progesterone interacts with the progesterone receptor (PR) to regulate multiple functions in the uterus, ovary, and breast [1–3]. Progesterone-like synthetic progestins are widely used clinically in combined estrogen/progestin hormone therapy (HT) for postmenopausal women and oral contraceptives (OC). These synthetic progestins can be classified based on structural features and also differentiated by steroid receptor selectivity. For example, natural progesterone and some of its derivatives, such as the 19-norprogesterones (e.g., trimegestone), lack androgenic activity [4]. In contrast, progestins derived from testosterone, such as the commonly used norethindrone acetate (NETA), retain a partial androgenic effect [4]. Differences also exist in the ability of these progestins to modulate other steroid hormone receptors, such as the mineralocorticoid, glucocorticoid, and estrogen receptor [5].

HT and OC use has been linked to an increased relative risk of both arterial and venous thrombosis [6–9], e.g., blood clots in the coronary arteries that cause heart attacks, clots in the deep veins of the extremities, and pulmonary emboli. The exact mechanism(s) by which sex hormones increase the risk for thrombosis has not been fully elucidated. However, several components of the thrombotic process are known to be modulated by sex hormones, including the blood coagulation pathway that generates the fibrin network component of the clot [10]. Normal blood coagulation involves

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a delicate balance between procoagulant and anticoagulant mechanisms [11,12]. Numerous clinical studies have shown HT and OC to modulate the plasma levels of many of the individual proteins in the pathway, possibly altering the balance to a more prothrombotic state in at least some patients [8,10,13–15]. Most of the effects of HT and OC on coagulation are thought to be derived from the estrogen component. While the potential effect of progestins on blood coagulation and thrombosis risk has gained more attention recently, there are still relatively few reports of clinical studies that were designed to look for progestin-specific effects.

Two essential plasma anticoagulants that were reported in clinical studies to be affected by HT and OC treatment are tissue factor pathway inhibitor (TFPI) and antithrombin (AT). TFPI, which inhibits the catalytic activity of the tissue factor/factor VIIa complex, is the primary regulator of the initiation phase of blood coagulation [16]. Various animal models of vascular injury have demonstrated that reduced endogenous TFPI is associated with enhanced thrombosis [17–20], and more recently, the importance of TFPI in man was confirmed by reports that individuals with low levels of TFPI have an ∼2-fold increased risk of venous thrombosis [21] and myocardial infarction [22]. Multiple clinical studies have shown that OC and HT use in women reduces TFPI plasma levels by ∼15–35% [15,23–27]. However, little is known of the effect that progestins specifically have on TFPI.

AT is a protease inhibitor that regulates the activity of thrombin and several other coagulation enzymes [28]. Complete absence of AT is incompatible with life, and individuals with partial deficiencies have an increased incidence of venous thrombosis [28]. Many clinical studies have reported an ∼5–15% reduction in plasma AT in women taking OC and HT [8,14,15,29–31]. In a recent placebo-controlled randomized trial of HT, a significant difference in AT levels was observed in women taking unopposed estradiol versus combined estradiol plus cyclical progestin, suggesting that the progestin may modulate AT plasma levels [13].

The objective of the present study was to first determine whether progestins can modulate AT and TFPI in an animal model, and if so, then to use the model to compare various progestins. The rat is routinely used in various models of arterial and venous thrombosis and to assay progestin activity using the uterus as the primary endpoint. Prolonged exposure of rats to diethylstilbesterol has been reported to alter coagulation protein levels in plasma [32], indicating that sex hormones can modulate coagulation in the rat. Therefore, we examined the effect on AT and TFPI levels in ovariectomized female rats treated with 17α-ethyinylestradiol (EE), NETA (a progestin commonly used in HT and some OC preparations), and two newer progestins from different structural classes, namely trimegestone (TMG) [33,34] and drospirenone (DSP) [35]. The data indicates that NETA has different properties compared to TMG and DSP in this rat model, which appear to reflect the estrogenic properties of the compound.

2. Materials and methods

2.1. Materials

NETA and 17α-EE were purchased from Sigma Chemical Co., (St. Louis, MO). Drospirenone and ICI-182,780 were synthesized by Discovery Synthetic Chemistry and Medicinal Chemistry, respectively (Wyeth Research, Pearl River, NY). Trimegestone was obtained from Aventis (Bridgewater, NJ). Human factor VIIa, factor X, and factor Xa were purchased from Enzyme Research Laboratories (South Bend, IN). Rabbit thromboplastin with calcium was from Sigma Chemical Co., and S-2765 was from DiaPharma Group, Inc. (West Chester, OH). Normal rat pooled plasma was prepared in house from 15 healthy, intact female rats and stored at −80 °C in small aliquots for up to 6 months.

2.2. Animals

Female Sprague–Dawley rats (Charles River Laboratory) were used under an approved Wyeth Research Animal Care and Use Committee protocol. Sexually mature rats (body weight 250–300 g) were ovariectomized by the vendor, placed on a casein-based diet (Purina Laboratory Rodent Diet 5K96C, Richmond, IN), and provided water ad libitum. Animals were assigned to treatment groups (n = 10-12 per group) such that group body weight means did not differ significantly and variances were equal (JMP software, SAS Institute, Cary, NC). Beginning 11–13 days after ovariectomy, the animals were dosed for 3 or 7 days with the indicated doses of EE either alone or combined with NETA, TMG, or DSP or with progestin alone, administered once daily orally by gavage in 2% Tween 80/0.5% methylcellulose vehicle. ICI-182,780 was administered once daily by subcutaneous injection in 10% ethanol/corn oil.

2.3. Plasma and tissue collection

Blood collection was performed approximately 24 h after the last treatment. Animals were anesthetized with ketamine (75 mg/kg)/medetomidine (0.5 mg/kg), and citrated blood was collected from the abdominal aorta without stasis using a 20 ga. angiocatheter. The first ∼1 ml of blood was discarded and 4.5 ml of blood was drawn into a syringe containing 0.5 ml of 3.8% sodium citrate. Blood was centrifuged at 2500 × g for 15 min (23 °C). Citrated plasma was collected, frozen in small aliquots, and stored at −80 °C until assay. Blood samples were rejected if they had a fibrin clot of any size or were grossly overcitrated (total volume ≤4 ml), and all plasmas were processed and frozen within 90 min of collection. Typically, 10 or more good quality plasma samples were obtained per treatment group.

Tissues for RNA isolation were collected from anesthetized rats immediately following blood collection. The heart, left kidney, and right lung were removed by standard surgical techniques. Because of reports that tissue factor